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Searching for long-term trends in prehistoric manuring practice. $\delta^{15}N$ analyses of charred cereal grains from the 4th to the 1st millennium BC

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Current concepts of prehistoric manuring are founded on limited and mainly circumstantial evidence, giving rise to much ambiguity with respect to the onset of systematic use of manure to enhance cereal production. This paper reports carbon (C) and nitrogen (N) contents and isotopic compositions ($\delta^{13}C$, $\delta^{15}N$) of charred grains of naked barley, emmer and spelt dating to the first four millennia of early agriculture in Denmark. The $\delta^{15}N$ values ranged from c. 0.5‰–5.5‰, 0.5‰–6.0‰ and 1.5‰–8‰ for spelt, emmer and naked barley, respectively. This study represents the until now most comprehensive investigation of long term trends in $\delta^{15}N$ values of charred cereal grains, which previous research have proposed as an indicator for prehistoric manuring practice. Our study suggests a long-term (3900–500 BC) decrease of manuring intensity in emmer cropping. Conversely the long-term (2300 BC – AD 1) trend for naked barley cropping displays a more distinct and significant increase (+2‰) in grain $\delta^{15}N$ values, reflecting an increased manuring intensity with an average $\delta^{15}N$ value of as high as 6‰. We interpret this trend as indicating the initiation of a more intensive and systematic manuring practice associated with cultivation of barley in the Early Iron Age (500 BC- 0). Although the isotopic signal ascribed to manuring was (somewhat) variable, the relative manuring effect was detected throughout the chronological continuum being investigated. Further, we observed that the conventional sample pre-treatment (acid-base-acid) induced an average $\delta^{15}N$ offset of 0.7‰ (pre-treated sample > non pre-treated sample). This has not previously been reported. Methodological advancements are needed to remedy this issue and provide consensus about appropriate pre-treatment of grain samples from archaeological sites. We conclude that N-isotope analysis of charred cereal grains constitutes a new and direct source of information about prehistoric manuring practice.

1. Introduction

The introduction of agriculture constitutes a radical and much debated change in human subsistence and life (Harlan, 1992; Price, 1995; Fischer et al., 2007; Hadjikoumis et al., 2011). The modes of production within a society often relate intimately to other societal or structural changes, for which reason the development, nature and different constituents of prehistoric of agriculture are important to examine in detail (Bogaard, 2004). Here we focus on the long term development of prehistoric manuring practice using stable isotope analysis of Danish findings of charred cereal grains.

A predominant opinion concerning the introduction of more intensive and systematic management of livestock and animal manure in Scandinavia is that such manuring practice occurred during the Bronze Age (Engelmark, 1992; Gustafsson, 1998; Robinson et al., 2009; Grabowski, 2011). Others have argued that the beneficial effect of manuring was acknowledged and utilised in Western Europe at an even earlier stage (Bakels, 1997). Still, the issue is debatable, mainly due to ambiguities in the interpretations of conventional archaeobotanical evidence (Lagerås and Regnell, 1999; Bogaard, 2004).

Manuring is known to be a critical factor in plant production and manuring practice could therefore constitute a key element in

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discerning different prehistoric agricultural regimes. Manure has a decisive effect on crop productivity and in areas with limited resources it could represent the difference between survival and surplus. Thus, the onset of systematic addition of animal manure to permanently cultivated fields would constitute a fundamental change in the nature of prehistoric agriculture.

Within recent decades, the attention of archaeobotanical research has been directed towards the diverse and more complex nature of crop husbandry (Bogaard, 2005). An overall agricultural regime may very well embrace a variety of underlying layers of differential modes of plant production and manure management. At present, it remains a challenge to discern how a specific assemblage of charred grains and weed seeds relate to different modes of manure management.

Recent studies have shown that the isotopic composition of nitrogen in plants is sensitive to manuring with regards to intensity and duration. Several interdisciplinary isotope studies demonstrate that 1) manure application exerts a critical control on grain, chaff and straw δ^{15}N (Bol et al., 2005; Bogaard et al., 2007; Fraser et al., 2011; Kanstrup et al., 2011) and 2) charring does not distort the isotopic composition of cereal grains (Bogaard et al., 2007; Kanstrup et al., 2012). This study examines to what extent analysis of ^{15}N abundance can be applied to archaeobotanical charred cereal remains and utilized as a direct source of information about the development of prehistoric plant production.

The applicability of the crop isotope approach was evaluated in relation to our understanding of agricultural development in Denmark, according to archaeological evidence and the environmental record. Being mainly empirical and explorative, our study was based on isotope analysis of a large collection of charred archaeobotanical cereal grains. Due to the novelty of the approach, the methodological issue of chemical sample pre-treatment was also addressed. This study represents the first comprehensive attempt to apply light stable isotope analysis (C and N) on a large assemblage of archaeobotanical material from a relatively small and well defined area (Denmark) and roughly covering 4000 years of agricultural history in order to elucidate long term manuring practice.

2. Material and methods

2.1. Archaeobotanical remains and sampling

The focus in this investigation was on three common early agricultural cereal types: emmer (*Triticum dicoccum*), spelt (*Triticum spelta*) and naked barley (*Hordeum vulgare, var. nudum*). The response of animal manuring on yield and isotopic composition in these cereals is well-known and is not cereal type specific (Kanstrup et al., 2011). Archived charred archaeobotanical cereal remains from Denmark were found via available published listings and catalogues, and via the open internet index of the Department of Environmental Archaeology and Conservation – Moesgård Museum (http://www.arkaeologi.dk/naturvidenskab/) (Robinson et al., 2009; Robinson, 1994, 2003). The geographical distribution of the samples included in this study is presented in Fig. 1. Generally, the preservation of the grains was very good. Optimally at least ten grains (although exceptionally single grain analyses and five grain samples were also included) were selected in order to execute a bulk isotope analysis.

While grains of naked barley have a very distinctive appearance, it is well recognized that grains of different wheat species including emmer and spelt are very similar and therefore difficult to separate with certainty (Hillman et al., 1996). Fortunately, the morphologically very distinctive glume bases of emmer and spelt are typically present in archaeobotanical assemblages from Denmark because the glume wheat species were generally stored as spikelets (Henriksen, 1992; Robinson, 2000; Møbjerg et al., 2007). For this reason, great emphasis was put on 1) selecting grains from finds that were dominated by one type of glume bases, 2) matching type of grains and glume bases, and 3) based on morphology, selecting only the most typical emmer or spelt grains. Appendix A provides information about the archaeobotanical samples included in the study.

2.2. Radiocarbon dating

Part of the samples in this study had previously been ^{14}C dated and these dates were obtained via literature, databases or personal communication. The remaining samples in this study (27) were ^{14}C dated in collaboration with the AMS ^{14}C Dating Centre at Aarhus University following standard procedures (Skovhus Thomsen, 1989; Philippsen, 2010). The raw radiocarbon determinations were presented in radiocarbon years (BP) in accordance with international conventions (Stuiver and Polach, 1977). These samples were used to test for effects of chemical pre-treatment effect and when searching for overall continuous temporal trends in the δ^{15}N values. Calibrated dates (BC) were used in the correlation of isotope results with main cultural historical periods and the assignment to overall pre-defined agricultural regimes (see below). Calibrations were performed in OxCal v4.1.7 (Bronk Ramsey, 2001) using atmospheric data from Reimer et al. (2009). See Appendix B for further information about samples and the calibration of the radiocarbon determinations.

2.3. Chemical pre-treatment

A comparison between a non- and a corresponding pre-treated subsample was conducted on 31 grain samples in order to investigate whether the widely used pre-treatment procedure intimately associated with radiocarbon dating influence the isotopic (C and N) composition of the samples. A subsample of 10 crushed and homogenized grains was analysed without any prior chemical pre-treatment while a subsequent subsample was pre-treated with a classic Acid-Base-Acid (ABA) pre-treatment following the standard procedure at the AMS ^{14}C Dating Centre at Aarhus University (Philippsen, 2010; Kristiansen et al., 2003). In principle this pre-treatment builds on the protocol suggested by Olsson (1976). In order to minimize the loss of material, especially during the base step, washings with distilled water between each step was avoided. In detail, the sample material was exposed to: 1) 1 M HCl for 1 h at 80 °C, 2) 1 M NaOH at 80 °C for 3 h after discarding the HCl. Very dark samples were treated once more with 1 M NaOH for 1 h, 3) 1 M HCl for approx. 16 h at room temperature (approx. 20 °C), 4) three washes in demineralised water, and 5) drying at 80 °C. The difference in isotopic composition, due to the chemical treatment was presented as the offset (pre-treated subsample minus non pre-treated subsample).

2.4. Isotope analysis

Each grain sample was crushed into a fine powder by hand in an agate mortar ensuring low contaminating grinding. Samples were dried at 60 °C prior to weighing. A subsample of 2.8–3.2 mg was weighed into tin capsules, packed and submitted for analyses. For approximately every five samples, a replicate was included to check measurement precision and the homogenization of samples. Concentrations of total C (C_{conc}) and total N (N_{conc}) and ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were analysed by combustion using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Crewe, Cheshire, UK) at the UC Davis Stable Isotope Facility, Davis, CA, USA. Isotope ratios are reported as δ^{13}C values (‰) referenced to $^{13}C_{VPDB}$ and δ^{15}N values (‰) referenced to $^{15}N_{AIR}$ following conventional practice within

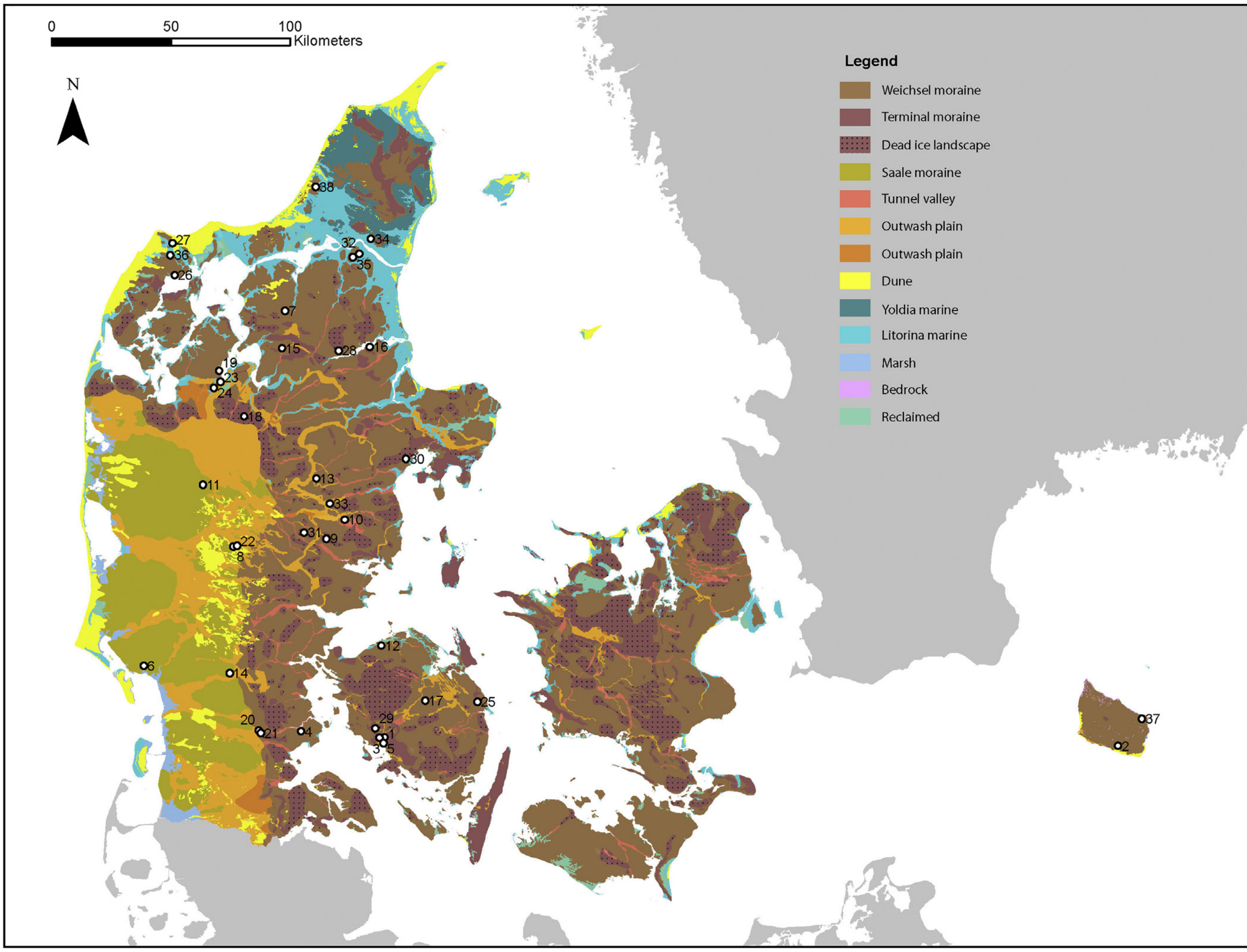

Fig. 1. Spatial distribution of the sites ($n = 38$) providing archaeobotanical samples for stable isotope analysis in this study. The number denotes site number which is the entry in Appendix A. Main geological landscape characteristics are shown on the background map.

isotope measurement and notation (Fry, 2006). Laboratory standards (±1 SD of the values obtained during the analysis are here reported following the reference value given by the laboratory) used for calibrating isotope data included nylon (−27.81‰ ± 0.033 and −9.77‰ ± 0.126), bovine liver (−21.69‰ ± 0.092 and 7.72‰ ± of 0.241), glutamic acid (37.63‰ ± of 0.034 and 47.6‰ ± of 0.156) and peach leaves (−26.12‰ ± of 0.055 and 1.95‰ ± of 0.128). These were calibrated against the IAEA standards USGS-40 (δ^{13}C: −26.389‰), USGS-41 (δ^{13}C: +37.626‰), IAEA-N1 (δ^{15}N: 0.4‰) and IAEA-N2 (δ^{15}N: 20.3‰) for C and N respectively. The analytical precision (±1 SD) of the check standard was better than 0.2‰ for δ^{13}C and 0.3‰ for δ^{15}N.

A total of 130 C- and N-isotope measurements were performed in the study (Table 1). The standard deviations of replicate measurements ($n = 16$) averaged 0.03 (ranging from 0 to 0.1) for δ^{13}C and 0.12 (ranging from 0 to 0.3) for δ^{15}N respectively. A few obvious outliers were detected, of which the Frydenlund sample (FHM5026X4571) was discarded from the subsequent data analysis since both δ^{13}C, N_{conc} and C/N diverged. Two additional samples (VMÅ2490X490 and ÅHM4473X263) had relatively low N_{conc} and C_{conc} but were included in the further analysis and interpretation. No other outliers based on the δ^{13}C − and δ^{15}N values (apart from the Frydenlund sample) were assessed as necessary to remove from the dataset.

2.5. *Statistics*

The effect of pre-treatment was tested by a paired Student's *t*-Test (significant if $\alpha = 0.05$). Simple linear regression was performed in order to examine continuous temporal development in δ^{15}N (significant if $\alpha = 0.05$). Correlation coefficient (r) and corresponding p-value were determined. SigmaPlot Statistics was used for these statistical operations. In order to examine any possible temporal trend in δ^{15}N values of successive agricultural regimes, the Kolmogorov–Smirnov test (KS-test) was applied when a sufficient number of samples were available (significant if $\alpha = 0.05$).

3. Interpretative framework

3.1. *^{15}N abundance as tracer of manuring*

Addition of animal manure to soil has been found to significantly increase the abundance of ^{15}N in the soil and especially in the grain, glume and straw fractions of cereal crops grown on the soil (Fraser et al., 2011; Kanstrup et al., 2011; Bogaard et al., 2007; Bol et al., 2005). The impact of manure depends on the rate, frequency and duration of application. For emmer, spelt and naked barley grown in the Askov long-term field experiment, Kanstrup et al. (2011) found that δ^{15}N in grains from unmanured and

Table 1
Results of isotope analysis. Total number of measurements was 130 of which 16 were duplicates. Double measurements are reported as the average followed by 1 SD in parentheses. Site numbers refer to Fig. 1 and Appendix A. Grains originated either as single grains (s) or in bulk samples (b) and were with (Y) or without (N) acid-base-acid (ABA) pre-treatment before analysis.

Site #	Sample ID	Origin	ABA	C (%)	N (%)	C/N	δ^{13}C (‰)	δ^{15}N (‰)
1	FHM5026X4571	b	N	41.8	1.52	27.6	−27.84	4.68
2	BMR948AC54	s	Y	60.6	3.53	17.2	−23.17	5.04
2	BMR948AC54	b	N	45.1	2.26	19.9	−26.23	5.03
3	FSM50X12676	b	Y	60.2	3.27	18.4	−22.91	3.11
3	FSM50X12676	b	N	47.9	2.20	21.8	−24.11	3.29
3	FSM50X12678	b	Y	59.7	3.11	19.2	−24.65	3.79
3	FSM50X12678	b	N	47.5	2.51	18.9	−24.56	3.66
3	FSM50X39706	b	Y	59.4	2.53	23.5	−25.03	3.40
3	FSM50X39706	b	N	46.3	2.21	22.0	−25.07	3.56
3	FSM50X39708	b	Y	59.2	2.93	20.2	−24.80	5.56
3	FSM50X39708	b	N	44.7	2.43	18.4	−24.80	5.51
				(1.3)	(0.08)		(0.01)	(0.08)
3	FSM50X39768	b	Y	64.2	3.08	20.8	−24.46	2.38
3	FSM50X39768	b	N	50.3	2.69	18.7	−24.52	2.25
3	FSM50X30094	s	Y	38.1	2.04	18.7	−24.52	3.42
3	FSM50X30094	b	N	47.5	2.44	19.5	−24.59	3.43
4	HAM1017Pr104	s	Y	61.3	3.85	15.9	−21.62	3.18
4	HAM1017Pr104	b	N	47.0	2.30	20.4	−24.01	6.19
5	FSM3527X4922	b	N	42.6	2.74	15.5	−23.97	5.44
6	ESM1658JP1	b	Y	65.7	3.67	17.9	−24.53	3.95
6	ESM1658JP1	b	N	54.7	3.38	16.2	−24.61	3.45
7	VMÅ2405X490	b	Y	60.7	3.31	18.3	−24.78	2.86
7	VMÅ2405X490	b	N	26.9	1.52	17.7	−24.57	1.88
8	HEM4026X94 nb	b	Y	64.6	2.98	21.7	−24.79	4.13
				(3.7)	(0.19)		(0.02)	(0.18)
8	HEM4026X94 nb	b	N	57.1	2.86	20.0	−24.77	2.83
8	HEM4026X515 nb	b	Y	67.6	3.24	20.8	−24.05	3.80
				(0.1)	(0.01)		(0.06)	(0.06)
8	HEM4026X515 nb	b	N	58.1	3.02	19.3	−24.10	3.20
8	HEM4026X491	b	Y	65.1	2.94	22.1	−24.94	3.57
8	HEM4026X491	b	N	56.0	2.70	20.8	−24.86	2.57
8	HEM4026X343	b	Y	64.5	2.96	21.8	−25.27	5.32
8	HEM4026X343	b	N	55.5	2.74	20.3	−25.20	4.44
8	HEM4026X732	b	Y	67.2	3.24	20.7	−25.57	3.44
8	HEM4026X732	b	N	47.5	2.75	17.3	−22.97	4.82
8	HEM4026X515 e	b	Y	66.0	3.56	18.5	−24.79	2.21
8	HEM4026X515 e	b	N	55.2	3.11	17.7	−24.84	1.82
8	HEM4026X94A37 e	b	Y	65.3	3.58	18.3	−23.66	1.86
8	HEM4026X94A37 e	b	N	51.2	2.94	17.4	−23.66	0.57
8	HEM4026X385	b	Y	62.4	3.82	16.3	−25.20	2.92
8	HEM4026X385	b	N	57.0	3.71	15.4	−25.14	1.81
9	HOM1509X30	b	Y	64.2	4.43	14.5	−25.42	2.12
9	HOM1509X30	b	N	60.5	4.19	14.4	−24.78	1.50
9	HOM1509X11	b	Y	63.4	4.10	15.5	−23.11	4.84
				(0.5)	(0.01)		(0.07)	(0.28)
9	HOM1509X11	b	N	55.5	3.79	14.6	−23.11	4.04
9	HOM1509X9	b	Y	62.4	4.03	15.5	−24.20	3.52
9	HOM1509X9	b	N	59.0	4.08	14.5	−24.26	1.53
10	SBM1194X10	b	Y	62.6	3.12	20.1	−23.3	6.07
10	SBM1194X10	b	N	46.6	3.06	15.2	−23.15	2.28
11	HEM4086X518 nb	b	Y	66.2	3.54	18.7	−25.16	3.98
11	HEM4086X518 nb	b	N	59.0	3.38	17.5	−25.02	2.68
11	HEM4086X518 s	b	Y	64.1	3.27	19.6	−23.70	1.34
				(0.8)	(0.03)		(0.01)	0.03)
11	HEM4086X518 s	b	N	57.0	3.00	19.0	−23.68	0.49
12	FSM1701huset nb	b	Y	62.5	3.29	19.0	−23.19	7.18
12	FSM1701huset nb	b	N	52.5	3.10	17.0	−23.29	6.47
12	FSM1701huset e	b	Y	60.8	4.02	15.1	−22.78	1.87
12	FSM1701huset e	b	N	49.5	3.63	13.7	−22.88	1.51
13	SIM82008X133	b	Y	67.4	4.22	16.0	−24.17	4.65
13	SIM82008X133	b	N	60.3	4.18	14.4	−24.17	3.87
14	HBV1275X530	b	N	46.5	1.98	23.4	−24.49	5.09
14	HBV1275X532	b	N	44.5	2.02	22.1	−25.44	4.08
14	HBV1275X533	b	N	42.2	2.00	21.1	−24.18	4.53
14	HBV1275X534	b	N	49.6	2.33	21.3	−24.64	5.15
				(0.1)	(0.01)		(0.01)	(0.03)
14	HBV1275X539	b	N	45.4	1.96	23.2	−24.92	4.41
				(0.7)	(0.08)		(0.08)	(0.23)
14	HBV1275X568	b	N	50.2	2.25	22.4	−25.31	2.43
14	HBV1275X569	b	N	49.2	2.31	21.3	−25.35	2.10
14	HBV1275X570	b	N	44.3	2.10	21.2	−25.52	2.74
				(6.1)	(0.32)		(0.01)	(0.08)
15	VSMG771X37	b	Y	64.5	3.83	16.8	−24.22	3.64
15	VSMG771X37	b	N	52.3	3.44	15.2	−24.27	2.71
16	ÅHM6022X6	b	N	46.8	2.75	17.0	−24.33	1.86
17	OBM2815X90	b	N	48.1	2.20	21.9	−23.22	2.83
17	OBM2815X92	b	Y	62.8	3.08	20.4	−23.38	2.85
17	OBM2815X92	b	N	51.8	2.80	18.5	−23.18	1.36
18	SMS731nb	b	Y	60.5	3.16	19.2	−22.91	5.47
				(2.7)	(0.13)		(0.07)	(0.11)
18	SMS731AX390 nb	b	N	60.1	3.14	19.2	−25.51	2.72
18	SMS731s	b	Y	62.0	2.91	21.3	−22.39	7.00
18	SMS731AX390 s	b	N	53.6	2.78	19.3	−22.41	5.57
19	SMS449Aa54	b	N	51.0	2.30	22.2	−24.28	3.48
20	HAM2957X755	s	Y	63.1	3.79	16.6	−24.36	2.57
20	HAM2957X755	b	N	49.3	2.60	19.0	−24.90	2.20
20	HAM2957X1143	s	Y	64.4	2.89	22.3	−24.92	4.26
20	HAM2957X1143	b	N	51.7	2.58	20.0	−25.35	4.05
21	HAM4940X17	b	N	46.9	3.10	15.1	−23.65	5.03
21	HAM4940X42	b	N	52.6	2.92	18.0	−25.27	2.88
22	HEM4357X538	b	N	50.4	2.71	18.6	−24.91	2.85
23	SMS270Aa632	b	N	47.8	3.12	15.3	−23.36	6.31
24	SMS722Ac17	s	Y	64.3	3.47	18.5	−24.36	8.87
24	SMS722Ac17	b	N	53.2	2.73	19.5	−24.55	6.90
				(1.4)	0.10)		(0.04)	(0.01)
25	OBM8935X132	b	N	41.4	2.19	18.9	−23.00	3.23
26	THY3750, 202	s	N	45.4	2.36	19.3	−22.97	4.30
26	THY5011X6 nb	s	N	50.7	2.61	19.5	−24.24	2.99
26	THY5011X6 e	s	N	49.8	3.67	13.6	−22.48	5.22
				(0.1)	(0.08)		(0.03)	(0.21)
26	THY3750, 147	s	N	40.1	2.16	18.6	−22.60	5.47
				(0.9)	(0.03)		(0.02)	(0.08)
26	THY3750, 85	s	N	44.4	3.07	14.5	−21.11	5.39
27	THY3718, 1549	b	N	54.2	3.01	18.0	−24.03	4.04
				(1.5)	(0.10)		(0.07)	(0.24)
28	ÅHM5754X3	b	N	47.9	3.14	15.3	−24.07	4.71
29	FSM1625X1027	b	Y	65.2	2.99	21.8	−23.73	1.82
29	FSM1625X1027	b	N	52.3	2.58	20.3	−23.77	1.93
				(0.3)	(0.01)		(0.02)	(0.10)
30	FHM4862X104	b	Y	58.3	2.90	20.1	−23.26	6.02
30-	FHM4862X104	b	N	36.9	2.20	16.7	−23.26	5.34
31	HOM1892X555	b	N	49.6	3.48	14.2	−22.93	8.04
32	ÅHM4473X9204	b	Y	67.5	3.17	21.3	−23.21	4.44
32	ÅHM4473X9204	b	N	52.9	2.92	18.1	−23.27	4.36
32	ÅHM4473X8656	b	Y	63.4	3.37	18.8	−23.70	6.75
32	ÅHM4473X8656	b	N	48.4	2.70	17.9	−23.54	6.37
32	ÅHM4473X263	b	Y	67.5	2.58	26.2	−23.47	6.29
32	ÅHM4473X263	b	N	17.7	0.80	22.1	−23.57	5.74
33	SBM 983P10	s	Y	62.6	3.12	20.1	−23.32	6.07
33	SBM 983P10	s	N	50.9	2.72	18.7	−23.63	5.01
34	FHM1790CBS	b	N	57.6	2.69	21.5	−23.47	6.71
35	ÅHM3844X3985	s	Y	60.5	3.43	17.6	−22.43	6.31
35	ÅHM3844X3985	b	N	50.4	3.02	16.7	−23.92	5.29
36	THY2960X895	b	N	46.1	2.02	22.9	−24.45	8.13
37	BMR1639 nb	b	N	48.8	2.14	22.9	−24.12	6.63
				(0.4)	(0.01)		(0.04)	(0.01)
37	BMR1639 e	s	Y	57.3	3.89	14.7	−22.89	2.07
37	BMR1639 e	b	N	47.7	2.61	18.3	−23.39	1.93
38	VHM151/1982	b	N	52.9	3.30	16.1	−21.97	5.24
				(0.1)	(0.02)		(0.01)	(0.22)

intensively manured treatments differed by 8.8‰. Other related studies embedded in modern field experiments have reported positive offsets in cereal grain δ^{15}N ranging 4–9‰ (Fraser et al., 2011; Bol et al., 2005). Thus an intentional and intensive use of animal manure will leave a distinct imprint on the N isotopic composition of cereal grains that reflects a combination of the historic and contemporary manure addition ahead of cereal grain harvest.

Grains from unmanured long term experimental plots serving as reference treatments have yielded δ^{15}N values below 4‰, with the

majority of the references being below 2.5‰ and in Danish studies below 2‰ (Fraser et al., 2011; Kanstrup et al., 2011; Bol et al., 2005). Cereals subject to intensive and systematic manuring have been suggested to be delineated by grain $\delta^{15}N$ values above 6‰ (Fraser et al., 2011). Manuring is probably the most obvious anthropogenic reason for elevated $\delta^{15}N$ values, but also clear-felling in forest eco systems has been suggested to result in somewhat increasing $\delta^{15}N$ values (Högberg, 1997).

The effect of initiating intensive and systematic manuring has recently been examined showing short-term increases of $\delta^{15}N$ ranging from approx. 1–3.5‰ (Fraser et al., 2011). The conclusions were not unambiguous since site-specific conditions and annual variations were confounded in this study. A field situation in which manuring ceased in 1871 provides some information on the long term residual effect of previous manuring. Ten years after the last addition of manure, $\delta^{15}N$ values of hulled barley had dropped from 6 to 4‰ (Fraser et al., 2011).

Currently there is solid evidence that grain $\delta^{15}N$ values are not distorted by charring (Bogaard et al., 2007; Kanstrup et al., 2012; DeNiro and Hastorf, 1985; Aguilera et al., 2008). We consequently assert that the nitrogen isotopic composition of charred grains, retrieved from archaeological sites, is a suitable indicator of prehistoric manuring practices.

3.2. Definition of agricultural regimes

We assessed that the number of samples available for this study may impose some constraints in the interpretation of the isotope evidence and in the resolution possible to obtain. For this reason, we chose to operate with three major agricultural regimes within the long continuum of agricultural history being investigated here. The definition of the regimes was based on an evaluation of the general archaeological evidence and the environmental record (e.g. archaeobotanical remains). We assumed that the isotopic differences due to manuring intensity in such a broad outline would be large enough to reveal consistent temporal trends despite a certain degree of variation in general. In this way we can study whether isotope evidence aligned with the overall agricultural development. The archaeobotanical samples were grouped according to the following agricultural regimes:

Regime I) The first regime dates from 3900 BC to 2800/2300 BC and represents the earliest evidence of agricultural activities in Southern Scandinavia beginning with the Funnel Beaker Culture. At the beginning of this period, traces of farming are very scarce, and it is not until around 3500 BC, that a substantial agricultural impact can be recognized based on for instance the pollen evidence. From this period onwards, traces of ard tilling are also regularly found beneath megalithic monuments, but the character of the fields is uncertain (Thrane, 1991). There are indications of a further intensification of agriculture and expansions of settlements after 3100 BC (Madsen, 1982; Jensen, 1994). The archaeobotanical evidence shows cultivation of mainly of emmer, followed by naked barley. Other, less common crops include einkorn, bread wheat, spelt and hulled barley. Spelt is very scarcely represented (Robinson, 2003, 2000).

Settlements are concentrated within well-defined areas in a landscape still largely characterized by forests. The sites are found on most soil types with a preference for sandy loamy soils and rarely on the nutrient poor sandy outwash plains of Western Jutland. The early settlements are small, but during the period we see a development of causewayed enclosures and large settlement complexes with intense activity traces covering several hectares (Madsen and Jensen, 1982).

Regime II) Around 2800 BC, the Single Grave Culture/Middle Neolithic B (MN B) marks a very significant change in settlement and land use patterns, indicating a new agricultural regime. The changes occur most dramatically in Jutland, while the East Danish region experiences a more gradual transition (Skaarup, 1985; Nielsen, 1993; Sørensen, 1995). Consequently, the beginning of MN B in eastern Denmark can be considered as a continuation of the previous land use practices and the beginning of regime II is here set to 2300 BC.

The pollen evidence indicates a general opening of the landscape with a beginning expansion of heath on sandy soils. This expansion continues gradually over the following millennia (Odgaard, 1994; Odgaard and Rasmussen, 2000; Andersen, 1998). An increased emphasis on animal husbandry has been proposed, but because of the lack of fauna material, the evidence remains circumstantial. Indicated by archaeobotanical evidence, a dramatic change in cultivation practice seems to take place during this regime. Naked barley is clearly the most common crop in the beginning of the period. Gradually emmer, and later spelt, increase in importance and all three species appear commonly and often together especially during the Bronze Age (1800–500 B.C.). At some sites from the Bronze Age and onwards though, there appears to be a reliance on more uncommon grain types like bread wheat and hulled barley. A general increase in the number of cultivated species on the sites is seen at the same time, probably indicating the emergence of a more flexible and opportunistic approach to agriculture than earlier. New species that appear during the Bronze Age include millet, gold of pleasure, oats, flax and rye. In some cases, these species most probably occurred merely as weed components (Robinson, 2000, 2003; Andreasen, 2009; Jensen and Andreasen, 2011).

The extensive settlements largely disappear and long houses, generally situated individually in the landscape, come to dominate the settlement pattern. Some houses reach considerable sizes of up to 500 m^2 in the Late Neolithic and Early Bronze Age (2300–1100 BC). Around 1500 BC, stalling of animals is introduced, which has been suggested to indicate a new manuring practice. However, the prevalence of stalling in general and its economic significance is still debated (Gaillard et al., 1994; Rasmussen, 1995; Barker, 1999).

Regime III) Around 600/500 BC, another significant change in land use pattern occurs, which we use to define the third agricultural regime. The transition is still poorly dated and may be gradually occurring over centuries from around 800–500 BC. In the present study, the boundary has been set at 500 BC. One of the most distinct changes is the appearance of Celtic Field systems, which quickly became widespread and indicate a significant change in landscape organisation and agricultural practices (Hatt, 1949).

The environmental evidence shows an overall continued expansion of settlement and land use and a reduction of forests. Also, in terms of cultivated plants, there are significant changes. Concurrent with a continual increase in the number of cultivated species compared to the Bronze Age (species like pea and horse bean for instance, now appear for the first time), there is a general decline in the importance of wheat. This also means that spelt, as well as emmer, now becomes relatively uncommon. Generally, cultivation is no longer focused on naked barley, emmer and spelt. The cultivation of numerous species and the tendency of more pronounced and diverse species preferences on individual sites indicate the emergence of a certain degree of specialization within crop husbandry. Barley remains important throughout the period. The importance of hulled six row barley, however, increases relative to the naked variety and becomes the dominant barley type around 1 BC/AD 1 (Robinson et al., 2009; Jensen and Andreasen, 2011).

Settlements are concentrated and co-existence of several long houses becomes a frequent phenomenon, which is often described as the emergence of the village (Becker, 1982; Hvass, 1988; Rindel, 1999). The individual long houses are generally small with living quarters of approximately 30 m^2. Architecturally, they are based on a fixed model with living quarters and stall situated in each end of

the house. This organisation is maintained throughout the time span covered in the present study, i.e. until 1 BC/AD 1. However, over time, the long houses generally tend to become larger and accompanied by various auxiliary buildings and fences.

An overview of the charred grain samples included in this investigation is provided in Table 2, where cereal type and associated prehistoric periods as well as agricultural regimes are also accounted for.

4. Results

4.1. Influence of chemical pre-treatment

Grain weight loss as a result of the ABA pre-treatment ranged from approx. 30%–80% with an average of 43% (data not shown). There was no relationship between weight loss and the calculated isotopic offsets. The comparison of corresponding pre-treated and non-treated aliquots ($n = 31$) of bulk samples (10 grains) shoved a general tendency of pre-treated samples having higher $\delta^{15}N$ values (Figs. 2 and 3). Although this was not absolute, the difference was statistically significant ($P \leq 0.001$) according to a Student's Paired t-Test. The ABA pre-treatment did not have any systematic effect on $\delta^{13}C$, and apart from a few outliers, $\delta^{13}C$ offsets were minimal.

The $\delta^{15}N$ offset between non- and pre-treated subsamples was not related to the age of the sample, and sample age was not decisive for weight loss, $\delta^{13}C$, C_{conc} or N_{conc} either (Fig. 2). No systematic relationship between the offsets in concentration and in the isotopic composition was seen (Fig. 3). Due to the obvious difference in $\delta^{15}N$, it was clear that non- and pre-treated samples should be analysed separately when examining for any possible long term temporal development. One sample (SMS731AX390 nb) had relatively high offsets in both $\delta^{13}C$ and $\delta^{15}N$. Nevertheless, $\delta^{13}C$ and $\delta^{15}N$ values were well within the general range of the remaining data set. The difference could imply special diagenetic conditions for this sample. Since it is not possible to establish whether non- or pre-treated subsamples represent the best approximation of the *in vivo* isotopic composition, this obvious discrepancy could be due to the non-treated values being anomalous or to an increased sensitivity towards the chemical pre-treatment due to poor preservation.

4.2. Chronological and spatial distribution

An uneven chronological distribution was evident from the sampled archaeobotanical material (Table 2 and Appendix B). The

Table 2
Number of samples (total of 72) included in this investigation divided on cereal type, associated prehistoric periods[a] and defined agricultural regimes (see text). Number in parenthesis denotes the number of sites providing the samples. More sample information is provided in Appendix 1.

Prehistoric period		Agricultural regime	Naked barley	Emmer	Spelt
FBC	3900–2800 BC	I	1 (1)	7 (2)	–
MN B	2800–2400 BC	I	1 (1)[b]	1 (1)[c]	–
LN	2300–1700 BC	II	9 (5)	5 (3)	1 (1)
EBA	1700–1000 BC	II	23 (15)[d]	2 (2)	4 (4)
LBA	1000–500 BC	II	4 (4)	–	2 (2)
EIA	500 BC– 0 AD	III	10 (8)	2 (2)	–

[a] The prehistoric periods were aligned with the Danish chronological framework commonly used when field excavation results are being reported. FBC: Funnel Beaker Culture, MN B: Middle Neolithic, LN: Late Neolithic, EBA: Early Bronze Age, LBA: Late Bronze Age, EIA: Early Iron Age (pre-roman Iron Age).
[b] This sample is archaeologically associated with early Battle Axe Culture.
[c] This sample is archaeologically associated with early East Danish Single Grave Culture.
[d] Kongehøj II alone provided eight of the EBA naked barley samples.

Table 3
Pooled $\delta^{15}N$ values (average ± S.E.) presented in Fig. 6 and number of samples ($n =$) providing the data.

Agricultural regime	Emmer		Naked barley	
	No ABA	ABA	No ABA	ABA
I	4.15 ± 0.49, $n = 8$	3.55 ± 0.37, $n = 7$	–	–
II	2.29 ± 0.66, $n = 7$	2.74 ± 0.56, $n = 5$	3.51 ± 0.24, $n = 36$	4.25 ± 0.46, $n = 16$
III	–	–	5.86 ± 0.47, $n = 11$	5.92 ± 0.38, $n = 6$

skewed spatial distribution of the sample sites reflects mainly sample accessibility (Fig. 1), but may also include a real bias associated with geographical differences in preservation and deposition conditions. The sampling sites are concentrated in Jutland and Funen, while finds are absent particularly from Zealand and the Southern Danish Isles. This can probably be contributed both to differences in excavation activities performed by different regional museums, as well as regional differences in soil types. Well preserved charred grains often tend to concentrate on sandier soil types, which are more prevalent in Western Denmark, particularly, in Jutland.

'The sampling process was prioritized to obtain chronological continuity. Apart from a hiatus in the Middle Neolithic B the period being investigated was covered in a satisfactory way. Among the analysed samples, the only two dated to the transitional period around 2800 BC belonged to the Eastern Danish region. Moreover, they exhibited some rather early dates and were consequently included in the first agricultural regime (I).

4.3. Single site $\delta^{15}N$ variation

From three sites (Sarup, Enkehøj and Kongehøj II) multiple samples of the same cereal type was obtained (Table 1). These samples show a large degree of isotopic variation with $\delta^{15}N$ values ranging from of 2.25‰–5.51‰, 2.57‰–4.82‰ and 2.10‰–5.09‰, respectively. The demonstrated within-site variation is important when interpreting isotope evidence and evaluating whether a single sample is representative of a given site. In the case of Kongehøj II, the multiple samples originated from the one house structure but the $\delta^{15}N$ values fell within two clearly distinct groups that could be separated spatially according to the construction and functional interpretation of the house (Fig. 4). In the northeastern corner of the house $\delta^{15}N$ values of the samples averaged 4.7‰ ± 0.5, $n = 5$ and the samples from a possible storage of grains in the middle room in the house resulted in $\delta^{15}N$ values averaging 2.4‰ ± 0.3, $n = 3$.

4.4. General trends in $\delta^{15}N$ values

There was a pronounced variability when relating $\delta^{15}N$ to radiocarbon years (Fig. 5). The majority of the samples had values between 2‰ and 6‰. The $\delta^{15}N$ values for spelt and emmer ranged from 0.5‰ to 5.5‰ and 6.0‰ respectively. Naked barley $\delta^{15}N$ values tended to be higher and ranged from c. 1.5‰–8‰. A linear regression for non pre-treated naked barley samples, which constituted the majority of the data ($n = 36$), was performed ($r^2 = 0.24$). This clearly showed that there was no simple linear correlation between age and $\delta^{15}N$ values. A supposed mixture of natural and cultural variation affecting the data could explain the variability in the sample material. Naked barley provided the highest $\delta^{15}N$ values (8 samples > 6‰), and these were mainly constrained to the latter part (Agricultural regime III) of the period

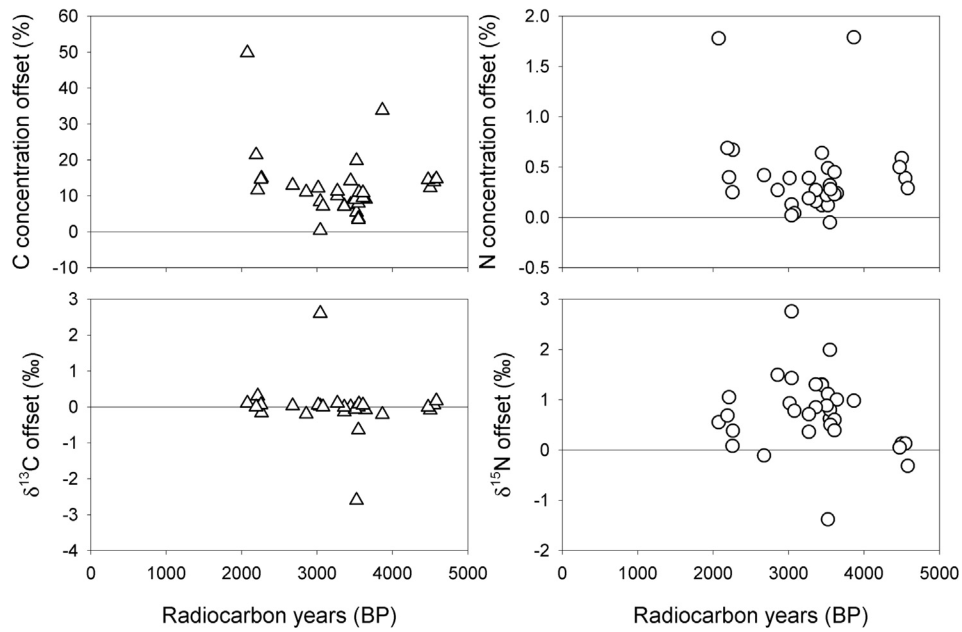

Fig. 2. The result of acid base acid (ABA) pre-treatment. Relationship between sample age (radiocarbon years) and offsets in concentration of C and N (top) and δ^{13}C and δ^{15}N (bottom). Offset = pre-treated − non pre-treated.

under investigation. Comparatively, only one emmer sample from the early phase of regime I (coinciding with the Funnel Beaker Culture) had a similar high δ^{15}N value above 6‰.

4.5. Long-term trends of agricultural regimes

In this part of the investigation the data from samples within each of the defined agricultural regimes are pooled excluding situations with less than five samples in total. Grouping together the δ^{15}N values of samples in this way resulted in two clear tendencies (Fig. 6). First, a tendency towards a decrease of c. 1.5‰ in emmer δ^{15}N values from agricultural regime I to II (3900–500 BC) could be seen. An increase of ~2‰ in δ^{15}N values of naked barley from agricultural regime II to III (2300 BC – AD 1) constituted the second tendency. The number of non pre-treated naked barley samples was sufficient to examine this tendency statistically with a KS test. The result provided statistical support for the bulked samples of naked barley in regime III (500 BC–AD 1) being significantly ($p = 0.001$) higher than the naked barley samples in agricultural regime II (2300 BC–500 BC). These temporal trends could be seen in both the non- and pre-treated samples.

5. Discussion

5.1. Methodological issues

During deposition, charred grains are exposed to soil-derived organic compounds, carbonates, and other inorganic ions that may contaminate the grains and potentially compromise their original isotopic signatures. Charred materials submitted for ^{14}C-dating are routinely cleaned by ABA (Olsson, 1976; DeNiro and Hastorf, 1985; de Vries and Barendsen, 1954; Hatte et al., 2001). Pre-treatment procedures draw upon the concept of soil organic matter being composed of fulvic acids, humic acids and humin with different ages and chemical compositions (Kristiansen et al., 2003; Stevenson, 1994). Within soil organic matter research, however, this concept has long been abandoned since the isolated fractions show little bearings on functional and structural properties of organic matter (Oades, 1989).

The ABA applied in this study caused an average weight-loss of 43%, but had no effect on the δ^{13}C value of the grains. In contrast, ABA introduced an average increase in δ^{15}N of 0.7‰ (excluding five outliers). DeNiro and Hastorf (1985) analysing prehistoric charred

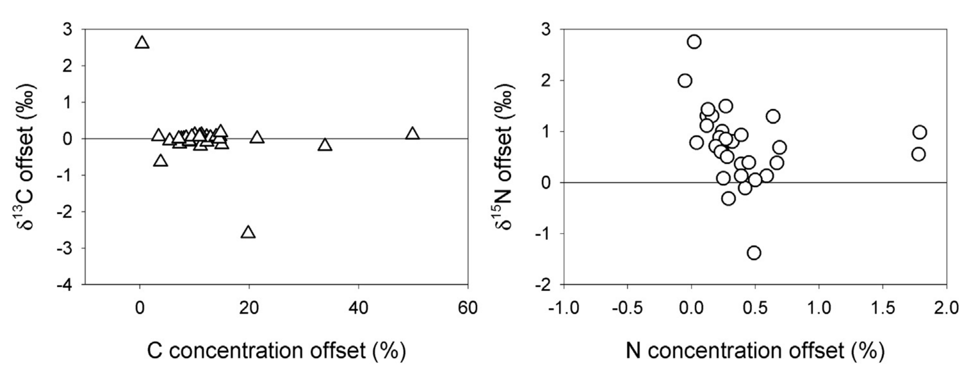

Fig. 3. Influence of acid base acid (ABA) pre-treatment. Relationship between concentration offset (C and N) and offset in isotopic composition (δ^{13}C and δ^{15}N). Offset = pre-treated − non pre-treated.

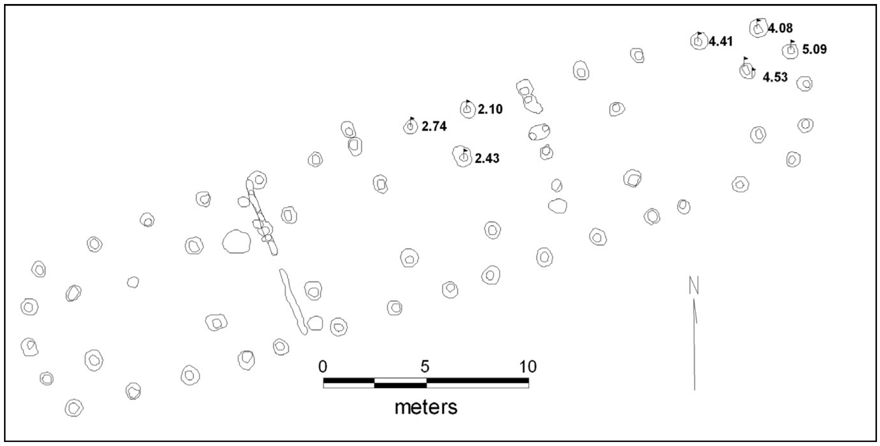

Fig. 4. Spatial distribution of the eight naked barley samples from Kongehøj II (site # 14), house K1 and the respective δ^{15}N values of eight (non pre-treated) separate archaeobotanical samples.

plant parts from Peruvian highlands found both increases (+0.8‰) and decreases (−0.6‰) in δ^{15}N due to chemical pre-treatment while changes in the δ^{13}C values were below 0.5‰. In our study the effect of ABA on isotope signatures and C- and N concentrations was not related to the age of the grain samples, indicating that the contamination level as judged by ABA was unrelated to the length of time in which the grains had been deposited in soil. The effect of ABA on δ^{15}N values contrasts a study of grains from Danebury Hillfort (Lightfoot and Stevens, 2012) in which just carbonates were removed with 6 M HCl and showed no systematic effect on grain ^{13}C and ^{15}N signatures. Our study does not reconcile the differential effects of ABA on the δ^{13}C and δ^{15}N values, but we conclude that the use of ABA in cleaning prehistoric charred grains before isotope analyses appears questionable. The development of more feasible cleaning procedures should be given priority in future research.

5.2. Sample representativity

Archaeobotanical analysis of the systematic sampling within the longhouse K1 at Kongehøj II delineated three major grain deposits within the house (Andreasen, 2011): 1) a storage of naked barley grains, situated in the eastern part of the house, interpreted as the living quarter and/or storage facility, 2) a close-by storage of wheat (not included in this investigation), and 3) a third large grain deposit of naked barley in the middle room (Fig. 4). Based on the archaeobotanical report, the two grain deposits dominated by naked barley could not be differentiated. Contextually the two assemblages differed and especially the function of the middle room was discussed in terms of whether it could have been a stable (Andreasen, 2011). The differences in δ^{15}N of up to 3‰ with an average offset of 2.3‰ (between 2.4‰ ± 0.3, n = 3 and 4.7‰ ± 0.5, n = 5) implied different manuring practice, where cereals were grown in different agrarian contexts, such as imbedded in different steps in a given field rotation system or under different scales of intensified agricultural production. We have previously shown that isotopic composition is similar for different grain sizes (Kanstrup et al., 2012), wherefore the two grain deposits are unlikely to represent differently sized grain fractions from the same harvest something, a conclusion substantiated by the archaeobotanical report (Andreasen, 2011).

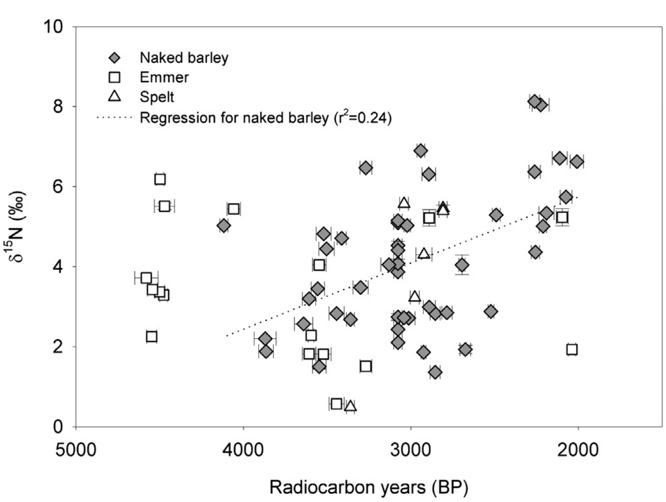

Fig. 5. δ^{15}N values of non pre-treated archaeobotanical samples of naked barley (n = 47), emmer (n = 17) and spelt (n = 7) in separate series in relation to sample age (radiocarbon years). The regression is for naked barley only. Vertical error bars denote 1 SD of double measurements. Horizontal error bars denote the uncertainty of the radiocarbon determination.

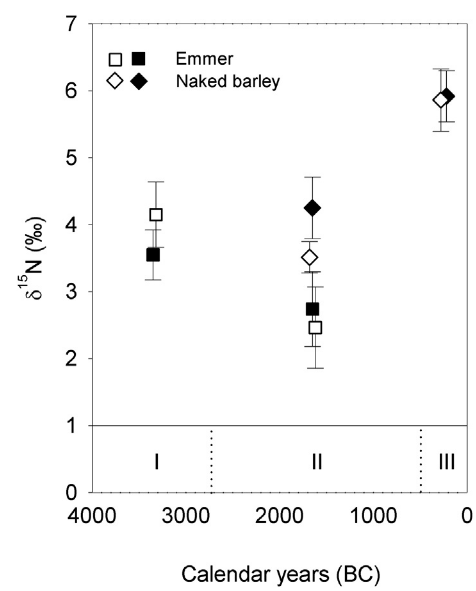

Fig. 6. Pooled δ^{15}N values in relation to agricultural regime (marked on the X-axis). The trend of non pre-treated (open symbols) samples and in pre-treated samples (solid symbols) was compared. Vertical bars denote standard error within the pooled data (n = 5–36, see also Table 3).

Based on architectural parallels the middle room in house K1 may have functioned as a stable although this was not unambiguously resolved based on the archaeological observations and the archaeobotanical report. Given the discussion about the function of the middle room in house K1 the low $\delta^{15}N$ values could indicate a secondary harvest kept for fodder rather than human consumption. Such findings could provide an alternative source of information to the more traditional archaeobotanical interpretations relying on weed seed compositions and the presence of weeds indicative of hay meadows assessed as an important fodder source and thereby associated with stables (Robinson, 2000). From the present study it remains clear that the combination of archaeobotanical analysis and isotope data can contribute with more qualified inferences about the plant production in prehistory and, perhaps also, assist in narrowing down the amount of possible interpretations.

The two distinct clusters of $\delta^{15}N$ values seen at the Kongehøj site can be interpreted as a situation where crops from different fields or harvests were not pooled, but more likely kept separate according to possible differences in consumption strategies or future uses, e.g. as seed material for the next growing season.

The large variation seen in $\delta^{15}N$ values within single sites and even between single features belonging to the same structure raises concerns about what the isotopic composition in a single sample from a site actually represents. In general, it would be advisable to collect multiple samples from sites in future crop isotope projects.

5.3. Agro archaeological perspectives

Cultural changes through time, in terms of both settlement types and grain storage practices, have influenced the spatial and chronological distribution of the samples. The large number of Late Neolithic and especially the Early Bronze Age samples are, to a large extent, due to a prevalent habit of storing grain in well protected features like sunken house floors and storing/depositing cropping material in pits (Møbjerg et al., 2007). The relatively large number of sampled sites from the early Iron Age on the other hand, can probably be attributed to a higher settlement density resulting in a larger number of excavated sites and archaeobotanical samples.

Chronological displacements in the cereal hierarchy also limit the ability to cover early agriculture all the way through by the three selected species. This is seen very clearly in the distribution of the spelt finds (Table 2), as the Danish finds of this species concentrate in the Late Neolithic and Bronze Age (Robinson et al., 2009; Robinson, 2003, 2000; Andreasen, 2009). A general replacement of naked barley by hulled barley, more or less from 1 BC/AD 1 (Jensen and Andreasen, 2011) was also of great importance to the investigation. Although the change is gradual, to some extent, it eventually leads to a total disappearance of naked barley during the 1st millennium AD. The species chosen for this study therefore put some constraints on the duration of the long term period possible to investigate. Other species or weed seeds could be included to supplement the data generated in this present study. This should be possible since the generalisation of "the manuring effect" has been shown to be similar for a wide range of cereal types (Bol et al., 2005; Fraser et al., 2011; Kanstrup et al., 2011).

The range in $\delta^{15}N$ values seen in the archaeobotanical remains of this study was, although wide and varied, well within isotopic values reported from modern field experiments, still existing traditional agrarian production sites and the few isotope investigations being published (Fraser et al., 2011; Lightfoot and Stevens, 2012). $\delta^{15}N$ values of plants have been shown to be sensitive to a range of different factors causing mixing and fractionations (Högberg, 1997; Robinson, 2001). Temporal developments or differences in $\delta^{15}N$ values need to be of a large or systematic order to ascribe them with any confidence to anthropogenic influences. Interestingly, there were some clear tendencies in the results despite the obvious variation.

Only a few samples fit cultivation without any previous additions of manure ($\delta^{15}N < 2‰$). The majority of the samples indicate manuring, although to a clearly variable extent with values ranging from 2 to 6‰. It should also be emphasised that indications of some degree of manuring was found throughout the entire prehistoric period being investigated. The intermediate $\delta^{15}N$ values could be associated with being the result of 1) incipient manuring, 2) extensive or more sporadic manuring practice and 3) residual effect of preceding manuring activities (Fraser et al., 2011). Some samples, predominantly from agricultural regime III, showed indication of more systematic and intensive manuring practice.

The general tendency of naked barley having higher $\delta^{15}N$ values compared to emmer, which was seen in agricultural regime II and III, agrees well with the recent findings from Danebury Hillfort (Lightfoot and Stevens, 2012). The range of $\delta^{15}N$ values of emmer and spelt was comparable, although spelt was only represented by relatively few samples.

Spelt samples were clearly confined to a relatively short period (ranging from 3547 ± 41 BP to 2808 ± 24 BP in this study), while the temporal scale for naked barley and emmer was more suitable to the main objective of the study. Looking at the proportional distribution of naked barley and emmer samples, the frequency of emmer samples declined through time, whereas the naked barley samples dominated in the latter part of the period under investigation. In this way, the samples included in this study agree well with the general archaeobotanical interpretation with glume wheat being gradually replaced by naked barley in the prehistoric cereal hierarchy (Robinson, 2003; Andreasen, 2009).

The nitrogen isotopic composition has been shown to reveal manuring practice (Fraser et al., 2011; Kanstrup et al., 2011). The present study therefore independently supported the archaeobotanical inferences of cereal hierarchical displacements. Emmer $\delta^{15}N$ tended to decline and naked barley $\delta^{15}N$ values increased significantly, which indicated matching displacements in crop nutritional attention.

The fact that naked barley in general yielded higher $\delta^{15}N$ values than glume wheat samples could contribute to the broader archaeobotanical discussion about a linkage between barley and an agrarian practice with more focus on plant nutrition issues. Such interpretations have so far been focused on hulled barley and based on, for instance, assemblage composition and ecological characteristics of associated weed seeds. This investigation indicates that naked barley also was manured to some degree (Engelmark, 1992; Gustafsson, 1998; Robinson, 2003). From the isotope analysis, it was not possible to conclude whether this linkage between naked barley and manuring was mainly due to a kind of cereal hierarchy with naked barley becoming more important and receiving more "nutritional attention".

Based on the crop isotope approach we find it unlikely that hulled barley in particular requires nitrogen-rich soils in order to produce an acceptable yield. Field experiments have previously shown an opposing trend, since the performance and isotopic response of naked barley in relation to the addition of animal manure resembled that of emmer and spelt (Kanstrup et al., 2011). Based on this we find it more likely that the nutritional demands of the three early agricultural cereal types were similar.

Despite the fact that non- and pre-treated samples are problematic to compare directly, it was clear (Fig. 6), that the two separate sample series showed the same general tendency in relation to agricultural regimes, although the number of pre-treated samples was not sufficient to execute valid statistical analysis. The pre-treatment induced $\delta^{15}N$ discrepancy of c. 0.7‰

did not distort the overall pattern emerging from the pooled data of emmer and naked barley showing temporal developments from agricultural regime I to II and from II to III respectively. The isotope analysis supports a concept of emmer receiving less manure going from agricultural regime I to II (3900–500 BC). This could be interpreted as being a result of a crop hierarchical displacement and marginalization of emmer, which complement the prevailing opinion among archaeobotanists (Robinson, 2003; Andreasen, 2009). The significant increase in $\delta^{15}N$ values (2‰) seen in the pooled naked barley samples representing the sequence of agricultural regime II–III (2300 BC – AD 1) on the other hand could be interpreted as indicating the initiation of a more intensive and systematic manuring practice clearly centred on barley cultivation from the beginning of the Iron Age (500 BC).

6. Conclusions

The effect of ABA pre-treatment on $\delta^{15}N$ found in this study constitutes a challenge for the future utilization of isotope analysis in connection with agro archaeological investigations. High $\delta^{15}N$ values have been found to be indicative of the use of animal manure and a tendency towards higher $\delta^{15}N$ values caused by ABA may introduce an over-estimation of manuring in prehistory.

From the set of multiple contemporary samples retrieved in features from one house structure, it is evident that even within the same cereal type large variations in $\delta^{15}N$ values may occur. At the Kongehøj II site, the isotope analysis contributed with independent evidence about probable differences (~3‰ in $\delta^{15}N$) in manuring practice, and that the harvested cereals were kept separate accordingly perhaps for different end-uses.

Based on the present study, we propose that the beneficial effect of manure was recognized and to some extent exploited throughout the whole period of early agriculture, although to varying degree.

We find that the long-term trends in prehistoric manuring practice as reflected in the isotopic signature of pooled data from emmer and naked barley samples, from the successive agricultural regimes I–II (3900–500 BC) and II–III (2300 BC – AD 1) respectively, substantiates previous inferences based on archaeobotany. The period of the earliest Iron Age (500 BC) yielded proportionally samples high in $\delta^{15}N$ (>6‰), indicating a more intense and systematic use of manure in cereal production.

We conclude that N isotopic analyses remain a promising approach when assessing prehistoric manuring practices. This direct signal of manure use seems to consolidate archaeobotanical inferences, but also convey new insight in support of a far more differentiated prehistoric crop husbandry practice. Considering the novelty of this approach a number of methodological issues, including chemical pre-treatment, needs to be addressed in more detail in order to develop standard protocols and facilitate the comparison of results from different studies. Furthermore, interpretations at this early stage should be validated by combining and comparing with other chemical and micro-morphological studies of, for instance, exposed palaeosols, as well as, contextualizing the isotope results to the more general knowledge based on the archaeological settlement evidence and the environmental record.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2013.04.018.

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